

Computational Design of TrkB Peptide Inhibitors and Their Biological Effects on Ovarian Cancer Cell Lines

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Abstract There are large numbers of different intracellular signaling pathways regulated by Tyrosine kinases (Trk) receptors. Trk receptors, especially TrkB, are also frequently overexpressed in a variety of human malignant tumors. In this study, we have computationally designed small peptide-based inhibitors of TrkB and investigated their effects on the proliferation and apoptosis of two ovarian cancer cell lines. Molecular docking of TrkB with its ligand and antagonist, BDNF and Cycloheximide B respectively, was carried out using HADDOCK program. A peptide library was constructed based on the critical residues involved in the TrkB binding site. After docking and optimization, two selected peptides were purchased and their effects on the viability and apoptosis of the cells were evaluated by performing MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) test and flow cytometry assay. Subsequently, the levels of expression and phosphorylation statuses of TrkB and its two downstream genes including MAPK3 and eIF4E were assessed

with western blot. We found that designed peptides effectively reduced TrkB, MAPK3 and eIF4E phosphorylation, reduced cell viability and induced apoptosis in the treated cells when compared to untreated cells. In conclusion, the BDNF/TrkB signaling is shown to be attenuated substantially in the presence of peptide inhibitors suggesting a strong inhibitory potential of the designed peptides for Trk family.

Keywords TrkB · Cancer · Small peptide · Docking

Introduction

The Tyrosine kinases (Trk) receptor family is one of the best-known class of transmembrane receptors that is necessary for development and survival of the mammalian nervous system. There are three most common types of Trk receptors including TrkA, TrkB, and TrkC which have different binding affinity to certain types of neurotrophins (Nakagawara 2001). Comparisons of their function indicate that TrkB and TrkC are the receptors for BDNF, NT4 and NT3 respectively, while TrkA is activated by nerve growth factor (NGF). These tyrosine kinase receptors are highly related to different intracellular signaling pathways regulating proliferation, cell survival, axonal and dendritic growth, synapse formation, cytoskeleton remodeling and membrane trafficking (Huang and Reichardt 2003). It has been widely demonstrated that Trk receptors activate both MAPKs and Akt, leading ultimately to further downstream signaling pathways for cell proliferation and development (Jang et al. 2007). However, many studies have reported that skewed population of neurons expresses TrkB, which suggests that this receptor plays a critical role in the regulation of neuronal development in vivo (Li et al. 2012).

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